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NZN's First Newsletter

The zebrafish research field is growing rapidly, with exciting developments taking place across institutions and disciplines. Yet, many of us continue to work independently, often with limited visibility into each other’s methods, ideas and experiences. This newsletter aims to change that.

By gathering experiences, methods, and ideas in one place, we can raise the quality of our work, avoid reinventing the wheel, and inspire one another. Whether you're a researcher, technician, veterinarian, or manager - this newsletter is for you.

It’s a space where we can share insights, ask questions, learn from each other, and build a stronger zebrafish community. Together, we can improve research, welfare, and facility operations.

Published quarterly, each issue will be a comprehensive edition featuring scientific insights, practical tips, community highlights,and an

overview of upcoming courses and conferences.

Most importantly, we hope **you** will contribute with your experiences and questions— the more voices we include, the stronger our community becomes. And if you know someone who might be interested, feel free to forward this newsletter to them!

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Nordic Zebrafish Network



Who are we?

This newsletter is brought to you by members of the Nordic Zebrafish Network (NZN), a collaborative initiative that connects zebrafish researchers, animal caretakers, and facility staff across the Nordic countries.

The NZN was founded following a workshop in Stockholm in 2023, where it became clear that improving zebrafish research and husbandry requires close collaboration between scientists and caretakers. By working together, we can enhance animal welfare, harmonize experimental procedures, and improve the reproducibility and significance of scientific data.

Our mission includes:

- Promoting the 3Rs (Replacement, Reduction, Refinement) in zebrafish research
- Facilitating knowledge sharing across institutions
- Developing standardized protocols to improve research quality and comparability

Whether you're a scientist, technician, veterinarian, educator, or simply passionate about zebrafish, you’re warmly welcomed into the NZN community

Together, we’re building a stronger, more unified zebrafish community - across borders and disciplines.



Husbandry and Welfare

A standardized Enrichment Divider for Zebrafish Tanks

The Zebrafish Core Facility at Karolinska Institutet has developed a new type of structural enrichment for zebrafish tanks -a standardized divider that mimics hanging vegetation found in the zebrafish’s natural habitat.

Unlike traditional plastic plants, this divider cannot be monopolized by dominant individuals, helping to reduce aggression. It’s made from inert, autoclavable plastic, poses no biosafety risk, and integrates seamlessly into existing tank systems and workflows.

The study which the zebrafish core facility published recently found no negative effects on growth, feeding, or reproduction, making this divider a safe and effective enrichment option for research facilities. The Divider will be commercialized by Tecniplast.

Read more

Water Quality as a Fondation

Stable water quality is the foundation of every zebrafish facility. It affects everything -from fish health and welfare to the reliability of research results. Even small fluctuations in key parameters can influence development, behavior, and immune response.

Different facilities solve this in different ways: some use automated systems for daily monitoring, while others rely on manual testing routines. Regardless of the method, routine documentation and quick response to deviations are essential.

Focus Topic: Nitrogenous Waste

This month, we highlight nitrogenous waste, a critical factor in zebrafish systems.

The main waste product from fish metabolism is ammonia, which also forms from decomposing feed and dead fish. Ammonia exists in two forms:

NH_3 (ammonia) – which is highly toxic

NH_4^+ (ammonium) – which is less harmful

The balance between these depends on pH and temperature. Even small increases in either can shift the equilibrium toward toxic NH_3. In healthy systems, nitrifying bacteria convert ammonia into nitrite (NO_2) and then into nitrate (NO_3). But if this process fails, nitrite can accumulate which poses a serious risk.

If ammonia or nitrite levels rise, it may be due to:

- Biofilter disruption (e.g. after cleaning or power outage)

- Low alkalinity, which affects bacterial survival
- Overfeeding or high biomass, increasing waste load
- Dead fish or uneaten feed decomposing in the tank

What you can do:

- Test Total Ammonia Nitrogen (TAN) and NO_2 frequently, especially after system changes
- Increase water exchanges to dilute waste
- Check and adjust alkalinity to support biofilter health
- Avoid overfeeding and remove uneaten food promptly
- Ensure stable pH and temperature to prevent NH_3 spikes

Did You Know?

A Pipette Pump Can Make Embryo Handling Easier

When working with zebrafish embryos, using a pipette pump instead of a traditional Pasteur pipette can significantly improve both precision and comfort. A pipette pump allows for smoother and more controlled aspiration and dispensing, which helps protect fragile embryos from mechanical stress. It also reduces hand fatigue during repetitive tasks which makes it especially useful during long sorting sessions. Unlike Pasteur pipettes, which rely on manual suction and can vary in consistency, pipette pumps offer better reproducibility and ergonomics. It's a small change that can make a big difference in both workflow and embryo welfare.

Buy here

Comparative transcriptomics coupled to developmental grading via transgenic zebrafish reporter strains identifies conserved features in neutrophil maturation

A recent study by Kirchberger et al. (2024) has provided a comprehensive analysis of neutrophil maturation in zebrafish, revealing significant parallels with mammalian systems. By generating transgenic zebrafish strains that represent distinct neutrophil differentiation stages, the researchers established a high-resolution transcriptional profile of neutrophil maturation. This approach allowed for the identification of key transcription factors, such as C/EBP-β, which are crucial for late-stage neutrophil development.

Cross-species comparisons between zebrafish, mouse, and human samples confirmed a high molecular similarity during the early stages of neutrophil maturation, while also highlighting species-specific gene signatures. Notably, applying the pan-species neutrophil maturation signature to RNA-sequencing data from human neuroblastoma patients revealed an association between metastatic tumor cell infiltration in the bone marrow and an overall increase in mature neutrophils.

These findings underscore the utility of zebrafish as a model organism for studying neutrophil development and function, with implications for understanding immune responses in both health and disease.

Read more

Updates on methods of humane killing of Zebrafish

A FELASA survey conducted in August 2022[1], involving 185 laboratory fish facilities across 23 countries, highlighted the need for updated guidelines on biosecurity, health monitoring, and humane euthanasia of laboratory fish. In response, a FELASA working group published practical guidance on humane killing of laboratory fish in April 2025[2].

The European Directive 2010/63/EU[3] (Annex IV) lists approved methods for killing animals, including fish. However, only one method is specifically relevant for zebrafish (*Danio rerio*). Other methods, such as percussion to the head, are impractical due to zebrafish's small size. Electrical stunning, commonly used in aquaculture, is unsuitable for adult zebrafish in laboratory settings. The survey revealed that the most commonly used euthanasia method for zebrafish is tricaine (MS222), followed by hypothermic shock. However, compliance issues were noted, as these methods often lack completion steps, such as brain destruction or permanent cessation of blood circulation.

The ideal euthanasia method should prioritize animal welfare, operator safety, environmental protection, and ease of use. The working group recommends hypothermic shock as a suitable method for adult zebrafish, provided the holding tank temperature is >26°C and the hypothermic bath is <2°C. While some fish may exhibit adverse effects during the procedure, the extent of distress or pain before loss of consciousness remains unclear. Hypothermic shock is not recommended for larval zebrafish. For zebrafish eggs and larvae (<5 days post-fertilization, dpf), electrical stunning is suggested, provided it induces loss of perception and consciousness within one second without causing physical injury (e.g., hemorrhage or spinal damage). However, this method requires specialized equipment, making it less practical than hypothermic shock.

Anaesthetic overdose, typically using tricaine, is widely used but not ideal for zebrafish due to adverse behavioral reactions. A more reliable and less aversive method involves buffered lidocaine hydrochloride (with or without 95% ethanol). This approach has shown minimal adverse reactions (as low as 2% in adults when ethanol is included)[4] and is effective for all developmental stages, including larvae, which absorb anaesthetics through their skin. The protocol for this method is detailed in the published recommendations.

Concussion or percussive blows to the head are rarely used but can be performed by experienced personnel, followed immediately by brain destruction. Decapitation, cervical dislocation, and spinal ablation are not recommended for any laboratory fish, including zebrafish.

The use of hypothermic shock has been added to the list of approved humane killing methods in the revised Annex IV of Directive 2010/63/EU, as per the EU Commission Delegated Directive 2024/1262 (13 March 2024)[5]. These amendment changes will take effect in EU Member States by 4 December 2025. In EEA countries like Norway, the updates require EEA approval before incorporation into national regulations.

Buffered lidocaine hydrochloride and hypothermic shock are now recognized as the most efficient and reliable euthanasia methods for adult zebrafish. These advancements mark significant progress in ensuring humane practices in laboratory fish research.

References

The Nordic Zebrafish Network is pleased to announce the launch of our **Community Corner Podcast** . In short episodes of around 10 minutes, we introduce members of our diverse community. Whether you are a scientist, educator, animal caretaker, or enthusiast, you are warmly invited to listen.

The Network serves as a hub for sharing experiences, best practices, and challenges in zebrafish research. By fostering collaboration, we aim to strengthen our community, avoid pitfalls, and accelerate scientific discoveries.

We are thrilled to kick off with two inspiring guests:

Associate Professor Louise von Gersdorff Jørgensen (University of Copenhagen),

A biologist specializing in fish immunology and aquaculture. Her research uses zebrafish to study immune responses in real time, advancing our understanding of host–pathogen interactions, vaccine development, and fish health.

Listen here

Dr. Chiara Zullian (Karolinska Institutet, Stockholm),

An EBVS® European Veterinary Specialist in Laboratory Animal Medicine. With international experience in veterinary pharmacology and laboratory animal medicine, Chiara focuses on aquatic animal health and is especially passionate about improving zebrafish care and welfare in research.

Listen here

Annual Advanced Zebrafish Husbandry Course at KI

This course provides comprehensive training in zebrafish Husbandry, small surgical procedures, rotifer culturing and cryopreservation/IVF. You can fin more information about the course on the link below. Sign up asap, few spots left!

10/11/2025 - 12/11/2025

Karolinska Institutet, Stockholm

Register here

Nordic Zebrafish Meeting 2025

This is our annual zebrafish conference, bringing everybody from the Nordics working on zebrafish together. Last year, almost all zebrafish labs from the Nordic Countries were represented, so join our conference this year! You will find more information and the link for registration below.

12/11/2025 - 14/11/2025

Elite Tower Caroline Hotel, Stockholm

Register here

NordicZebrafishNetwork

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